

# Rett syndrome: new clinical and molecular insights

In this review, we give a clinical overview of Rett syndrome (RTT), and provide a framework for clinical and molecular approaches to the diagnosis of this severe neurodevelopmental disorder. We also discuss issues that need to be considered in the management of RTT patients, and raise some of the challenges associated with genetic counselling.

## In brief

- Incidence of 1:10 000 female births by the age of 12 years
- Severe neurodevelopmental disorder affecting females
- Normal early development is followed by loss of fine and gross motor skills and communication
- Characteristic stereotypic hand movements in most affected individuals
- A number of clinical RTT variants
- Pathogenic mutations in the gene *MECP2* have been identified in up to 90–95% of classical RTT
- *MECP2* gene defects can give rise to non-RTT phenotypes
- Pathogenic mutations the genes *CDKL5* and possibly *Netrin G1* account for a small proportion of cases
- Disease mechanisms remain unclear
- Management is currently symptomatic

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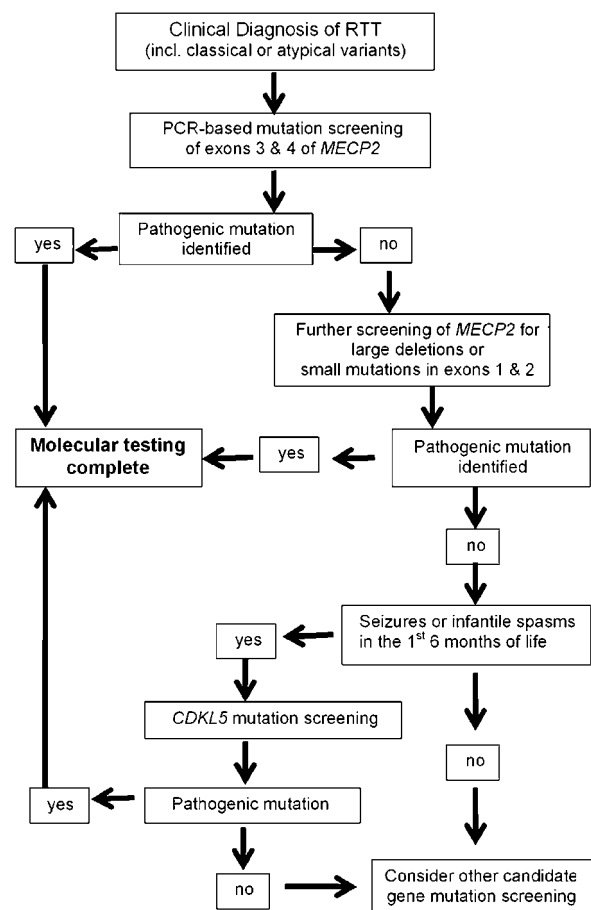
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**Figure 1** Genetic testing strategy for RTT. Mutation screening of *MECP2* exons 3 and 4 is recommended initially followed by exons 1, 2 and MLPA or other methods (to detect large deletions). If a pathogenic mutation is not found, *CDKL5* screening is recommended for patients with a severe seizure disorder in the first 6 months of life.

## Introduction

Rett syndrome (RTT; OMIM 312750) is a severe neurodevelopmental disorder primarily affecting females and has an incidence of 1:10 000 female births by the age of

12 years<sup>1</sup> making it one of the most common genetic causes of severe mental retardation (MR) in females. It is characterised by apparently normal development for the first 6–18 months of life followed by the loss of acquired fine and gross motor skills and the ability to engage in social interaction, and the development of stereotypic hand movements. There is a wide variability in the rate of progression and severity of the disease, and as well as the classical form of RTT there are a number of recognised atypical variants.<sup>2,3</sup>

Mutations in the X-linked gene methyl CpG-binding protein 2 (*MECP2*) have been found in of the majority of patients, and more recently mutations in two other genes, cyclin-dependent kinase like 5 (*CDKL5*)<sup>4–7</sup> and *Netrin G1*<sup>8</sup> have been identified in patients with a clinical phenotype that strongly overlaps with RTT (Figure 1).

### Clinical synopsis

Although it was initially believed that the recognisable features of RTT appear after an apparently normal prenatal, perinatal and early infancy period, more recent studies have clearly shown that even in the first 6 months of life the female RTT infant may display subtle behavioural abnormalities.<sup>9,10</sup> A general stagnation of development is followed by the loss of fine and gross motor skills, social interaction and intellectual functioning. A more definitive clinical picture evolves in stages over a number of years, culminating in motor deterioration and ultimate demise.<sup>11</sup> The Trevathan diagnostic criteria<sup>2</sup> were revised in 2001 at a satellite meeting of the European Paediatric Neurology Society,<sup>3</sup> which has helped in resolving inconsistencies and ambiguities in the categorisation of patients into classical or variant RTT (see Table 1).

It is now clear that females with RTT may have a much broader phenotype than originally described, with a number of variants now described which may be more or less severe than the clinical picture seen in classical RTT. Moreover, it is now known that there exist rare males with a severe neonatal-onset encephalopathy, with prominent breathing abnormalities.<sup>12–14</sup> In addition, a number of males with a phenotype comparable to females with classical or atypical RTT have been described, some of who also have a 47XXY karyotype,<sup>15–17</sup> whereas others are mosaic for severe *MECP2* mutations,<sup>18</sup> and still others with MR have *MECP2* mutations, which when seen in females are associated with only a much milder phenotype<sup>19</sup> (for a review see<sup>20</sup>).

### Diagnostic approaches

The diagnosis of RTT remains a clinical one, using criteria such as those outlined in Table 1. In addition, guidelines have been developed to facilitate more consistent descrip-

**Table 1** Revised diagnostic criteria for classical and variant RTT<sup>a</sup>

Classical RTT	
<i>Necessary criteria</i>	
1.	Normal prenatal and perinatal history
2.	Normal psychomotor development for the first 6 months
3.	Normal head circumference at birth
4.	Postnatal deceleration of head growth in most individuals
5.	Loss of purposeful hand skills between 6 months and 2½ years
6.	Hand stereotypies
7.	Evolving social withdrawal, communication dysfunction, loss of acquired speech, and cognitive impairment
8.	Impairment or deterioration of locomotion
<i>Supportive criteria</i>	
1.	Breathing disturbances during waking hours
2.	Bruxism
3.	Impairment of sleeping pattern from early infancy
4.	Abnormal muscle tone associated with muscle wasting and dystonia
5.	Peripheral vasomotor disturbances
6.	Progressive kyphosis or scoliosis
7.	Growth retardation
8.	Hypotrophic small and cold feet and/or hands
<i>Exclusion criteria</i>	
1.	Evidence of a storage disorder including organomegaly
2.	Cataract, retinopathy, or optic atrophy
3.	History of perinatal or postnatal brain damage
4.	Confirmed inborn error of metabolism or neurodegenerative disorder
5.	Acquired neurological disorder due to severe head trauma or infection
Variant RTT	
<i>Inclusion criteria</i>	
1.	At least three of the six main criteria
2.	At least five of the 11 supportive criteria
<i>Main criteria</i>	
1.	Reduction or absence of hand skills
2.	Loss or reduction of speech (including babble)
3.	Hand stereotypies
4.	Loss or reduction of communication skills
5.	Deceleration of head growth from early childhood
6.	Regression followed by recovery of interaction
<i>Supportive criteria</i>	
1.	Breathing irregularities
2.	Abdominal bloating or air swallowing
3.	Bruxism
4.	Abnormal locomotion
5.	Kyphosis or scoliosis
6.	Lower limb amyotrophy
7.	Cold, discoloured and usually hypotrophic feet
8.	Night time screaming and other sleep disturbances
9.	Inexplicable episodes of screaming or laughing
10.	Apparently diminished sensitivity to pain
11.	Intense eye contact and/or eye pointing

<sup>a</sup>Modified from Hagberg *et al*: *Eur J Paediatr Neurol* 2002; 6: 293–297.

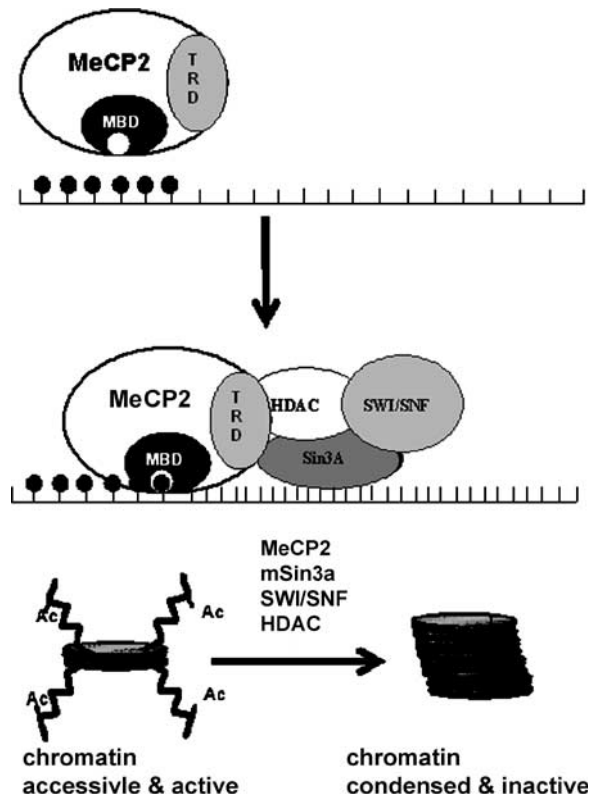
tion of clinical features.<sup>21</sup> The identification of a *MECP2* mutation can support a clinical diagnosis but should not be used as a basis for diagnosis. Further classification into

whether the individual has classical RTT or one of the recognised variants is of value when coupled with *MECP2* mutational findings in terms of allowing potential future genotype–phenotype correlations (see below).

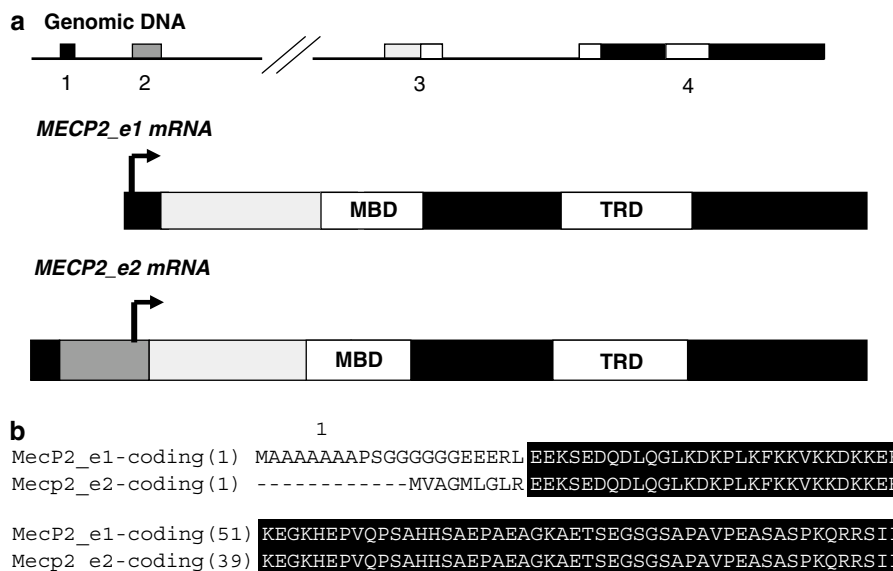
### Molecular and genetic basis of RTT Mutations in *MECP2*

*MECP2* is an X-linked gene that has two isoforms *MECP2\_e1* (*MECP2- $\alpha$*  or *MECP2* B form) and *MECP2\_e2* (*MECP2- $\beta$*  or *MECP2* A form), created by alternative splicing of exon 2 and the use of two alternative start codons. The resulting proteins are almost identical but have alternative N-termini (Figure 2). The MeCP2 protein has two major functional domains the methyl-binding domain (MBD), which binds specifically to DNA at methylated CpG's, and a transcription repression domain (TRD) that is responsible for recruiting other proteins that mediated transcription repression (Figure 3).<sup>22–26</sup> The *MECP2\_e1* isoform has only recently been described and is the most abundant of the two isoforms.<sup>27,28</sup>

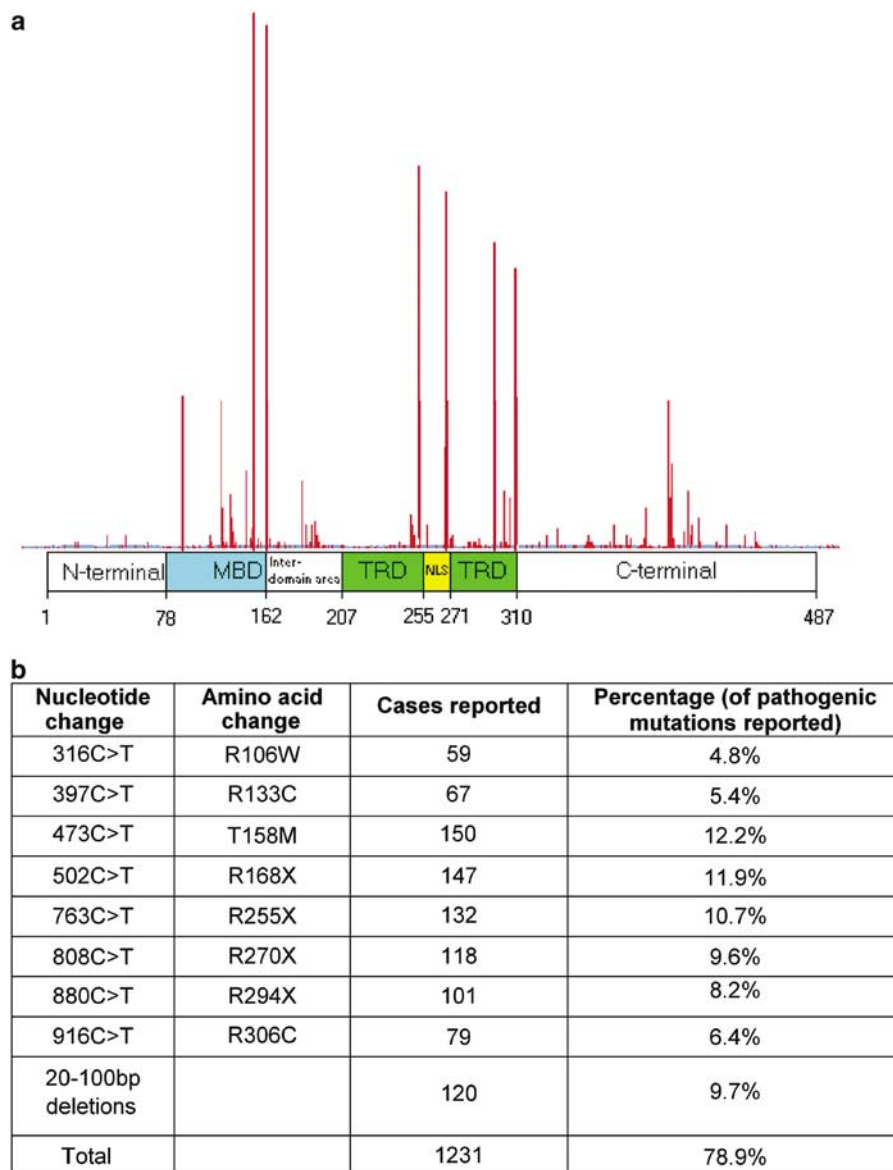
Mutations in *MECP2* were first reported in 1999,<sup>29</sup> and subsequent screening of RTT patients has shown that almost 90–95% of classical RTT have pathogenic mutations.<sup>30</sup> To date over 200 individual nucleotide changes which cause pathogenic mutations have been described (Figure 4, RettBASE; [mecp2.chw.edu.au](http://mecp2.chw.edu.au)<sup>31</sup> and [MeCP2.org.uk](http://MeCP2.org.uk)); however, the eight most commonly occurring missense and nonsense mutations account for almost 70% of all mutations, and small deletions associated with a deletion hotspot in the C-terminal region of the MeCP2 protein account for an additional 9% of pathogenic



**Figure 3** The mechanism of transcription repression by MeCP2. The methyl-binding domain (MBD) of MeCP2 binds specifically to methylated CpG sites on DNA, the transcription repression domain (TRD) then recruits several corepressor proteins. This results in the deacetylation and condensation of chromatin, which is now not accessible to the transcription machinery. Thus, genes in the region of DNA that MeCP2 binds are not expressed.



**Figure 2** The two *MECP2* isoforms. (a) Genomic structure of the two *MECP2* isoforms and the two alternative mRNA species produced. The arrows indicate the start codons for each mRNA. (b) An alignment of the first 100 amino acids of MeCP2\_e1 with MeCP2\_e2 indicating the differences in the N-terminus.



**Figure 4** Frequency of pathogenic mutations in *MECP2*. (a) The position and frequency of reported *MECP2* mutations in RettBASE. The numbers refer to the amino-acid position in the MeCP2 protein sequence. (b) Frequency of the most common mutations in RettBASE.

mutations. Mutations are found throughout the gene, and more recently large deletions (kilobases in size) that delete whole exons have also been identified in a proportion of patients who were previously considered to be mutation negative, and are more commonly found in individuals with classical (36%; 46 out of 128) than atypical (3%; seven out of 229) RTT cases.<sup>32–38</sup> On the other hand, pathogenic mutations involving exon 1 appear to be only rarely associated with RTT.<sup>30,37,39,40</sup> In almost all cases the mutations are *de novo*, and there is some evidence suggesting that in the majority of cases the mutation has arisen on the paternal X chromosome.<sup>41,42</sup>

More recently, whole duplications of the *MECP2* gene have been associated with severe X-linked MR with progressive spasticity, no or poor speech acquisition and acquired microcephaly,<sup>43</sup> which has been recapitulated in a mouse model overexpressing MeCP2.<sup>44</sup> Clinical descriptions of younger patients with duplications or with functional disomy of the Xq28 region have also noted severe hypotonia with or without the development of microcephaly, and interestingly cryptorchidism in males.<sup>45–47</sup>

It is important to note that the identification of a mutation in *MECP2* does not necessarily equate to a

diagnosis of RTT. *MECP2* mutations have also been found in other clinical phenotypes, including individuals with an Angelman-like picture,<sup>48,49</sup> nonsyndromic X-linked MR,<sup>50</sup> PPM-X syndrome,<sup>51</sup> autism<sup>52</sup> and neonatal encephalopathy.<sup>13,14,53</sup> Males with a neonatal encephalopathy phenotype generally have *MECP2* mutations, which when seen in females are associated with more severe RTT.<sup>13,14,53</sup> Males with a more RTT-like phenotype may also have an X-chromosome aneuploidy<sup>15–17</sup> or may be mosaic for a *MECP2* mutation.<sup>18</sup> Contrary to earlier studies<sup>19,48,50,52</sup> recent studies of large cohorts of autistic patients and males with nonspecific MR suggest that pathogenic *MECP2* mutations are only rarely observed in these groups of patients.<sup>54–56</sup>

Genotype–phenotype correlations have given conflicting results but generally truncation mutations tend to be more severe than missense mutations.<sup>57</sup> Genotype–phenotype correlations are complicated by variations in the severity scales used to describe phenotype, although this issue may be resolved if there is consistency in the descriptions used.<sup>21</sup> In addition, skewing of X-chromosome inactivation may modulate the clinical severity of the disorder, as was seen in a family in which a T158M mutation was seen in a mother who was clinically normal and had completely skewed X-chromosome inactivation favouring the normal allele, and in her daughter with clinically diagnosed RTT and at least one of her sons who died early in life from severe apnoea.<sup>12</sup>

Rather than being a global transcription repressor as initially suspected, MeCP2 may mediate the expression of a subset of specific targets in the brain. There is recent evidence that MeCP2 binds specifically to certain DNA sequences.<sup>58</sup> Several studies have indeed identified specific MeCP2 targets, including *Hairy2a*,<sup>24</sup> brain-derived neurotrophic factor (*Bdnf*),<sup>59,60</sup> *Dlx5*,<sup>61</sup> and *sgk*.<sup>62</sup> It is also worth noting that the observation that the features of RTT correlate with the timing of synapse development has led to the most recent hypothesis that MeCP2 may be responsible for controlling the expression of genes, which control the development and maintenance of synapses, although this remains to be proven.<sup>63,64</sup>

### Mutations in other genes

As stated above, a proportion of individuals with a clinical diagnosis of RTT do not appear to have mutations in the *MECP2* gene. In recent times mutations in two other genes, *CDKL5*<sup>4–7</sup> and *Netrin G*,<sup>8</sup> have been shown to be associated a phenotype that strongly overlaps with that seen in RTT.

In virtually all cases reported to date *CDKL5* mutations have been associated with an early onset seizure variant of RTT, the so-called Hanefeld variant.<sup>4–7</sup> Recently, evidence was presented, indicating that there may be a direct interaction between *CDKL5* and MeCP2<sup>65</sup> suggesting that

they are a part of a common pathway in the regulation of neuronal cell function, but it remains to be confirmed how this interaction contributes to the pathogenesis of the RTT-like phenotype. Moreover, it is apparent that mutations in this gene account for only a small subset of RTT patients.

Only one case of a *Netrin G1* mutation has been described<sup>8</sup> and so it remains to be established whether there is a direct causal link with RTT and this gene or whether it should be included as a part of systematic genetic testing of *MECP2* mutation-negative RTT patients.

### Management

#### Treatment and care

Unfortunately, there are currently no specific treatments that halt or reverse the progression of the disease, and so management is mainly symptomatic and individualised, focussing on aiming to optimise each patient's abilities. A dynamic multidisciplinary approach is most effective, with specialist input from dietitians, physiotherapists, occupational therapists, speech therapists and music therapists.<sup>66</sup> Attention needs to be paid to scoliosis (seen in about 87% of patients by the age of 25 years),<sup>67</sup> and the development of spasticity,<sup>68</sup> both of which can have a major impact on mobility, and to the development of effective communication strategies for these severely disabled individuals.<sup>69</sup> Psychosocial support for families is an integral part of the holistic approach to management, and parent support groups, such as the International Rett Syndrome Association (<http://www.rettsyndrome.org/>), the Rett Syndrome Research Foundation (<http://www.rsrf.org/>), and the Rett Syndrome Association UK (<http://rettsyndrome.org.uk/>) offer immense practical day-to-day support to families.

Pharmacological approaches to managing problems associated with RTT include melatonin for sleep disturbances,<sup>70</sup> several agents for the control of breathing disturbances, seizures and stereotypic movements,<sup>71,72</sup> and L-carnitine for general well-being.<sup>73</sup> RTT patients have an increased risk of life threatening arrhythmias associated with a prolonged QT interval,<sup>74</sup> and so avoidance of a number of drugs is recommended, including prokinetic agents (eg, cisapride), antipsychotics (eg, thioridazine), tricyclic antidepressants (eg, imipramine), antiarrhythmics (eg, quinidine, sotalol, and amiodarone), anaesthetic agents (eg thiopental and succinylcholine), and antibiotics (eg erythromycin and ketoconazole). In addition, careful evaluation for evidence of central autonomic function using noninvasive methods may be of value in identifying specific patterns of disturbance, and may ultimately lead to specific therapies for this sometimes very distressing set of clinical problems.<sup>75</sup>

Following the report of reduced CSF folate levels in four females with RTT,<sup>76</sup> Neul analysed CSF from an additional 76 individuals with RTT, but could not reproduce earlier

findings, and found that supplementation with folic acid did not lead to any noticeable clinical improvements.<sup>77</sup> It therefore remains to be established whether cerebral folate deficiency contributes to the pathophysiology of RTT.

### Genetic testing strategies

A recommended screening strategy (Figure 1) is to firstly screen exons 3 and 4 for mutations using PCR-based techniques such as denaturing HPLC and sequencing, as the majority of mutations are seen in this region. If no mutation is identified further screening of exons 1 and 2 is recommended. No pathogenic mutations have yet been identified in exon 2, whereas mutations in exon 1 appear to be rare in RTT patients. Large deletions would be missed by most PCR-based screening strategies, and so for individuals in whom no pathogenic mutations have been found by screening exons 1–4 additional screening for large deletions is recommended, which may be achieved by Southern analysis, quantitative PCR of genomic DNA or alternatively Multiplex Ligation Probe Amplification (MLPA), using a commercial MLPA kit that is specifically developed for *MECP2* (MCR-Holland).

Finding a pathogenic mutation in a RTT patient can be used to support the clinical findings in that individual. Most, but not all, pathogenic mutations are *de novo*, so it is recommended that parental DNA is screened for a mutation identified in a proband, and if the mother is found to have a mutation, analysis for skewing of X-chromosome inactivation may be of help in counselling the family. A number of rare polymorphisms have been seen in parents and unaffected siblings,<sup>78</sup> and available *MECP2* locus-specific mutation databases are valuable tools that can be used to distinguish such rare polymorphisms from a truly pathogenic variation.

Patients who are negative for *MECP2* mutations and who have a strong clinical diagnosis of RTT, particularly if there are early onset seizures, should be considered for further screening of the *CDKL5* gene. However, it should be noted that the identification of any *CDKL5* sequence variation should be treated with caution, and that testing of first-degree relatives would be essential to permit classification of any variation found.

### Genetic counselling

As pathogenic *MECP2* mutations in RTT patients are mostly *de novo* the recurrence risk is low, estimated empirically to be less than one in 300,<sup>79</sup> although gonadal mosaicism cannot be excluded.<sup>29,80</sup> The option of prenatal screening should be discussed in families with a proband having a pathogenic mutation. The precise type of mutation, its location, and the presence of skewing of X-chromosome inactivation may together help to a certain degree in prognostication of disease severity.<sup>66</sup>

### Conclusion

RTT is notable for marked variability in the severity and progression of the disorder and at least in the early stages can be difficult to diagnose for those not experienced in the disorder. Treatment remains focused on predicting and treating problems as they develop to improve the quality of life of individuals.

Despite the identification of mutations in *MECP2* in the majority of RTT patients the pathophysiological basis of the disorder remains unclear. Future research will focus on the identification and characterisation of the molecular pathways controlled by MeCP2, particularly focusing on those involved in the development and maintenance of synapses. It will be interesting to study the possible relationship between MeCP2 and CDKL5. It is hoped that through these combined approaches, specific therapeutic targets may be identified, which ideally would slow or even arrest disease progression altogether, and may yield insights into other neurodevelopmental disorders.

### Further reading

<http://mecp2.chw.edu.au> and <http://www.iscr.ed.ac.uk/mecp2/>

International Rett Syndrome Association (<http://www.rettysyndrome.org/>)

The Rett Syndrome Research Foundation (<http://www.rsr.org/>)

Rett Syndrome Association UK (<http://www.rettysyndrome.org.uk/>) and

Rett Syndrome Australian Research Fund (<http://www.nesher.com.au/rett/menu-02.htm/>)

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